Author's response to reviews

Title: Modification of additive effect between vitamins and ETS on childhood asthma risk by GSTP1 polymorphism: a cross sectional study

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Author's response to reviews: see over
Thank you for taking the time out of referee’s busy schedule to write to us.

Your feedback regarding my revised manuscript is greatly appreciated and has assisted us in improving it. I have made some corrections and clarifications in the manuscript after going over the referee’s comments.

The detail changes and reasons are summarized as below:

1. Table 1 and eTable 1 are hard to understand and some data are inconsistent. For example, the number of children enrolled in this study was 1111, and 245 children were excluded in eTable 1. However, in lines 111-113 stated that: 1,124 children (588 boys and 536 girls) were included in this study. The 232 children who had not answered the food questionnaire were excluded ----. The total number of the each parameter was inconsistent;

Thank you for your kind comment. Table 1 and eTable 1 were totally revised. I am very sorry about the errors in methods. I corrected subject number in methods. “Of the responders, 1,111 children (577 boys and 529 girls) were included in this study. The 245 children who had not answered the food questionnaire were excluded because of insufficient information about their calorie intake.”

The expressions of age and BMI were wrong; what do the “Continuous”, “ETS duration (min.)”, “Frequency of ETS” mean?
I corrected the expression of age and BMI in Table 1 and eTable 1. ETS duration (min) means “If your child was exposed to tobacco smoke more than once a week, how long was your child exposed to tobacco smoke?”. Frequency of ETS means “If your child was exposed to tobacco smoke more than once a week, how many times was your child exposed to tobacco smoke for a week?” We added these descriptions in methods. We would like to add the result of frequency of ETS in Table 1. Your understanding in regards to this would be very much appreciated.

I think the data of pulmonary function test is unreliable, please check it.

: Thank you for your comment. As your comment, we tested the data of pulmonary function test again. We changed the description of pulmonary function test to % predictive value.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total</th>
<th>ETS (+)</th>
<th>ETS (−)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1111</td>
<td>403</td>
<td>708</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.48±1.73</td>
<td>9.54±1.68</td>
<td>9.44±1.76</td>
<td>0.384</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>577/529</td>
<td>366/337</td>
<td>211/192</td>
<td>0.925</td>
</tr>
<tr>
<td>BMI</td>
<td>18.46±3.30</td>
<td>18.77±3.48</td>
<td>18.28±3.18</td>
<td>0.024</td>
</tr>
<tr>
<td>Parental history of asthma</td>
<td>38/875 (4.3%)</td>
<td>14/315(4.4%)</td>
<td>24/560(4.3%)</td>
<td>0.912</td>
</tr>
<tr>
<td>Parental history of AR</td>
<td>347/885 (39.2%)</td>
<td>109/322(33.9%)</td>
<td>238/563(42.3%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Parental history of AD</td>
<td>88/896 (9.8%)</td>
<td>28/323(8.7%)</td>
<td>60/573(10.5%)</td>
<td>0.384</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (≤ high school)</td>
<td>391/1090 (35.9%)</td>
<td>162/391(41.4%)</td>
<td>229/699(32.8%)</td>
<td>0.004</td>
</tr>
<tr>
<td>High</td>
<td>699/1090 (64.1%)</td>
<td>229/391(58.6%)</td>
<td>470/699(67.2%)</td>
<td></td>
</tr>
<tr>
<td>Household income</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10,000 Korean won)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 299</td>
<td>312/1055 (29.6%)</td>
<td>134/386(34.7%)</td>
<td>178/669(26.6%)</td>
<td>0.019</td>
</tr>
<tr>
<td>300–399</td>
<td>292/1055 (27.7%)</td>
<td>102/386(26.4%)</td>
<td>190/669(28.4%)</td>
<td></td>
</tr>
<tr>
<td>≥ 400</td>
<td>451/1055 (42.8%)</td>
<td>150/386(38.9%)</td>
<td>301/669(45.0%)</td>
<td></td>
</tr>
<tr>
<td>Paternal smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>293/1078 (27.2%)</td>
<td>34/394(8.6%)</td>
<td>259/684(37.9%)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
### Past smoker
- 263/1078 (24.4%)
- 36/394 (9.1%)
- 227/684 (33.2%)

### Current smoker
- 522/1078 (48.4%)
- 324/394 (82.2%)
- 198/684 (29.0%)

### Non-smoker
- 1075/1091 (98.5%)
- 382/391 (97.7%)
- 693/700 (99.0%)

### Maternal smoking
#### Past smoker
- 6/1091 (0.6%)
- 2/391 (0.5%)
- 4/700 (0.6%)

#### Current smoker
- 10/1091 (0.9%)
- 7/391 (1.8%)
- 3/700 (0.4%)

### Frequency of ETS (per week)
- ≤ twice: 85/215 (39.5%)
- 2-4 times: 59/215 (27.4%)
- ≥ 5 times: 71/215 (33.0%)

### Serum total IgE (kU/L)*
- 58.13±4.01
- 59.45±3.65
- 57.38±4.22

### Pulmonary function test*
- FVC (%): 89.34±1.13, 89.35±1.13, 89.33±1.14
- FEV₁ (%): 96.91±1.13, 96.86±1.13, 96.93±1.13
- FEV₁/FVC: 92.12±1.07, 91.99±1.07, 92.2±1.06
- FEF₂₅-₇₅ (%): 90.63±1.38, 91.89±1.31, 89.96±1.41

### Wheeze in previous 12 months
- 62/1069 (5.8%)
- 31/386 (8.0%)
- 31/683 (4.5%)

### Asthma diagnosis
- 110/1064 (10.3%)
- 50/390 (12.8%)
- 60/674 (8.9%)

AR, allergic rhinitis; AD airways disease; BMI, body mass index; ETS: environmental tobacco smoke.

*Values are mean ± SD or n (%).

2. About the GSTP1 (rs1695) polymorphisms, it is not only description in the paper, but also the results of some related data analysis, such as HWE, other genetic model and allele, should be added in the results.

: I can really appreciate your comment. We added the result of HWE in the results. **The distribution of GSTP1 polymorphism was in Hardy–Weinberg equilibrium (HWE). The number of three genotypes (AA, AG, and GG) of GSTP1 polymorphism is 597(62.6%).**
319(33.4%) and 38(4.0%), respectively. HWE P-value of this polymorphism is 0.569.”

We also tried to analyze other genetic model and allele in this study. Because small number of children with GG genotype, we were not able to fully analyze other genetic model and allele. Response table 1 shows these results. Thus we do not want to describe these results in this manuscript.

Response table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vitamin A</th>
<th>ETS</th>
<th>Caroten</th>
<th>ETS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype</td>
<td>Asthma diagnosis</td>
<td>Genotype</td>
<td>Asthma diagnosis</td>
</tr>
<tr>
<td></td>
<td>AA+AG</td>
<td>(n=596)</td>
<td>GG</td>
<td>(n=26)</td>
</tr>
<tr>
<td></td>
<td>Yes/No (%)</td>
<td>aOR* (95% CI)</td>
<td>Yes/No (%)</td>
<td>aOR* (95% CI)</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(%)</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>High</td>
<td>No</td>
<td>15/104(12.61)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>No</td>
<td>20/227(8.1)</td>
<td>0.97 (0.44 2.16)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Yes</td>
<td>9/70(11.39)</td>
<td>1.03 (0.42 2.55)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Yes</td>
<td>29/122(19.21)</td>
<td>3.13 (1.42 6.91)</td>
</tr>
<tr>
<td>Retinol</td>
<td>High</td>
<td>No</td>
<td>15/109(12.1)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>No</td>
<td>20/222(8.26)</td>
<td>0.91 (0.41 2.03)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Yes</td>
<td>18/61(22.78)</td>
<td>2.81 (1.27 6.20)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Yes</td>
<td>20/131(13.25)</td>
<td>1.64 (0.73 3.68)</td>
</tr>
<tr>
<td>Caroten</td>
<td>High</td>
<td>No</td>
<td>14/100(12.28)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>No</td>
<td>21/231(8.33)</td>
<td>0.88 (0.40 1.94)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Yes</td>
<td>11/68(13.92)</td>
<td>1.33 (0.56 3.17)</td>
</tr>
</tbody>
</table>
3. About the question 6, I mean that whether children with the AA genotype who had been exposed to ETS benefit from antioxidant supplementation, e.g., vitamin A or carotene, and the relative data should be added in the results.

: Thank you for your comment. In table 4, children who were exposed to ETS, low dietary vitamin A and retinol intake were more strongly associated with the risk of wheeze in the previous 12 months than were high dietary intake (vitamin A: aOR 4.43, 95% CI 1.51–12.96; retinol: aOR 5.15, 95% CI 1.63–16.25). In eTable 2, children with the AA genotype who had been exposed to ETS and had low intakes of vitamin A or carotene were at increased risk of asthma diagnosis compared with those with children with no ETS exposure and high intakes of vitamin A or carotene (vitamin A: aOR 4.44, 95% CI 1.58–12.52; carotene: aOR 3.15, 95% CI 1.15–8.63, eTable 1, Fig. 1). However, these exposures did not significantly elevate the odds of asthma diagnosis in children with AG or GG genotypes. Our study suggests that low vitamin A
intake increases susceptibility to the development of ETS-associated childhood asthma, possibly through decreased antioxidant capacity. Our data also show that the oxidative-stress-related gene GSTP1 may modify this association.

Although I totally agreed your comment, unfortunately, antioxidant supplementation was not investigated in this study. We hope that you understand our situation and greatly appreciate your patience. We described this limitation in discussion. Further studies on the preventive effect of antioxidant supplementation on children with AA genotype and exposure to ETS are needed.

Quality of written English: Needs some language corrections before being published

This manuscript was received proofreading again.

We hope the revised manuscript will better meet the requirements of the ‘BMC pulmonary medicine’ for publication. I thank you again for the constructive review by the reviewers.